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TITLE: Evaluation of MMX1902 as an Oral Treatment for Duchenne Muscular Dystrophy

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In the cardiac-focused study conducted, 10-week-old *mdx* mice were exercised at 15 m/min for 60 minutes 2 times a week for 10 weeks. Four different SQ treatment groups (n = 9/group) were evaluated – wild-type controls and three groups of *mdx* mice: vehicle (saline) and two MMX1902 doses (1.0, and 2.0 mg/kg/day). DMD-associated cardiomyopathy is a dilated cardiomyopathy and, as such, is marked by increased left ventricular volume and decreased ejection fraction with compensatory tachycardia. Following treatment and exercise, echocardiography showed daily MMX1902 treatment at the 2 mg/kg/day dose to reduce left ventricular end systolic volume, increase ejection fraction, and ameliorate tachycardia resulting in cardiac functional measures comparable to exercised wild-type control mice. Further, embryonic myosin heavy chain (eMHC) staining of the diaphragm showed a significant increase in eMHC positive muscle fibers with MMX1902 treatment, at both doses, supporting to the potential for MMX1902 treatment to stimulate and sustain regeneration even in the face of long-term, intensive exercise.

15. SUBJECT TERMS

Duchenne Muscular Dystrophy, renin, angiotensin

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Duchenne Muscular Dystrophy (DMD) is one of the most common and devastating genetic diseases of childhood, affecting approximately 1 in 3500 live male births. We have developed a small molecule agonist of the Mas receptor that has provided benefit in *mdx* mice, an animal model of DMD. This proposal seeks to: 1) test the oral efficacy of MMX1902 in *mdx* mice, 2) assess the ability of MMX1902 to provide benefit with delayed treatment, 3) evaluate the potential of MMX1902 to positively affect cardiac function, 4) optimize the synthesis of MMX1902 and produce GMP material, and 5) establish a complete ADME profile on the molecule.

2. KEYWORDS:

Duchenne Muscular Dystrophy, renin, angiotensin

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- **Specific Aim 1** - Oral MMX1902 Dose Optimization
- **Specific Aim 2 –** Delayed Administration of MMX1902
- **Specific Aim 3 –** Evaluation of Cardiac Function Following MMX1902 Administration
- **Specific Aim 4 –** Small Batch GMP Manufacture of MMX1902
- **Specific Aim 5 –** Pre-IND Meeting with FDA

What was accomplished under these goals?

• **Specific Aim 3 –** Evaluation of Cardiac Function Following MMX1902 Administration
In the cardiac-focused study conducted, 10-week-old *mdx* mice were exercised at 15 m/min for 60 minutes 2 times a week for 10 weeks. Four different SQ treatment groups (n = 9/group) were evaluated – wild-type controls and three groups of *mdx* mice: vehicle (saline) and two MMX1902 doses (1.0, and 2.0 mg/kg/day). DMD-associated cardiomyopathy is a dilated cardiomyopathy and, as such, is marked by increased left ventricular volume and decreased ejection fraction with compensatory tachycardia. Following treatment and exercise, echocardiography showed daily MMX1902 treatment at the 2 mg/kg/day dose to reduce left ventricular end systolic volume, increase ejection fraction, and ameliorate tachycardia resulting in cardiac functional measures comparable to exercised wild-type control mice (**Table 1**). Further, embryonic myosin heavy chain (eMHC) staining of the diaphragm showed a significant increase in eMHC positive muscle fibers with MMX1902 treatment, at both doses, supporting to the potential for MMX1902 treatment to stimulate and sustain regeneration even in the face of long-term, intensive exercise (**Figure 1**).

Table 1. Echocardiography Measure as **Percentage** of Wild-Type Mice

Measure	WT (%WT)	Vehicle (%WT)	MMX1902 1 mg/kg (%WT)	MMX1902 2 mg/kg (%WT)
Ejection Fraction	100±3.4*	89.1±2.9	95.3±3.2	98.2±2.8*
Heart Rate	100±2.5*	107.7±1.7	104.4±2.5	99.4±2.5*
End Systolic Volume	100±13.5*	150±14.6	130.5±10.6	108.6±9.8*
Long Axis Length Systole	100±3.5*	109±1.6	108.3±3.9	103.1±1.8*

*Values significantly different from vehicle treated *mdx* mice ($p<0.05$)
All values represent mean±SEM

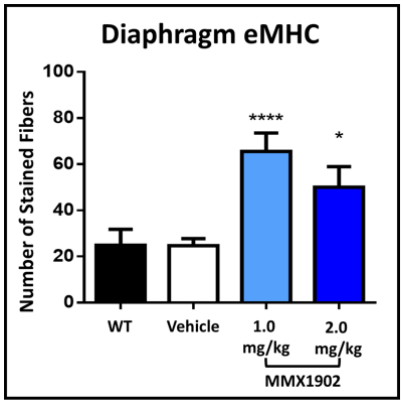

Figure 1. Following 10 weeks of intensive treadmill exercise and treatment. eMHC staining of the diaphragms of showed a significant increase in regeneration compared to vehicle treated *mdx.* *=P ≤ 0.05, ****=P ≤ 0.0001 in comparison with vehicle control.

• **Specific Aim 4 –** Small Batch GMP Manufacture of MMX1902
In order to prepare for the large-scale synthesis of MMX1902 under GMP manufacturing conditions, the synthetic route and conditions were transferred to Kemxtree Research Laboratories. Prior to initiation of this work, only 200 mg MMX1902 had been produced. By exploring a number of alternative reaction conditions. This led to the elimination of the only cryogenic reaction, a major synthetic bottle neck and cost-driver at scale-up, and the synthesis of 10.2 grams of MMX1902 at >98% purity.

What opportunities for training and professional development has the project provided?

• **Specific Aim 3 –** Evaluation of Cardiac Function Following MMX1902 Administration
During the course of this study, the team working on this study, Kevin Gaffney, Andrew Tiemann, Michael Weinberg, and Chris Meeks, all increased their familiarity with animal handling and increased or improved their skills at echocardiography acquisition and analysis and immunohistochemistry (IHC). Further, these individuals became proficient at the analysis of echocardiography tracings. These advances are evidenced in the data outlined in the previous section.

How were the results disseminated to communities of interest?

Nothing to Report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we have set out to advance the following *Specific Aims*:
• **Specific Aim 1** - Oral MMX1902 Dose Optimization
This study is currently underway and we are planning and have the data analysis of this study completed for the next reporting period.
• **Specific Aim 2 –** Delayed Administration of MMX1902
This study will start following the completion of the analysis of *Specific Aim 1* with the potential for the data analysis of this study to be completed for the next reporting period.
• **Specific Aim 4 –** Small Batch GMP Manufacture of MMX1902
During to the next reporting period, we will commence the stability testing on MMX1902.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

• **Specific Aim 3 –** Evaluation of Cardiac Function Following MMX1902 Administration
This study illustrates and confirms the use of our molecule in the treatment of cardiac deficits in individuals with Duchenne muscular dystrophy.
• **Specific Aim 4 –** Small Batch GMP Manufacture of MMX1902
The results of our scale-up of MMX1902 illustrates the scalability of this molecule and provides a route to the synthesis of large quantities of MMX1902 for stability testing, further pre-clinical animal studies, and, eventually, IND-enabling toxicology studies.

What was the impact on other disciplines?

• **Specific Aim 3 –** Evaluation of Cardiac Function Following MMX1902 Administration
The preliminary data obtained thus far illustrates the potential of our molecule to positively affect cardiac performance. Based on this data and literature on natural peptide activator of our receptor of interest, our molecule has the potential to treat patients with cardiac maladies beyond patients with Duchenne muscular dystrophy.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Based on our preliminary cardiac efficacy data, our program shows the potential to reduce cardiac pathology, a major cause of mortality in patients with Duchenne muscular dystrophy.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

• **Specific Aim 1 -** Oral MMX1902 Dose Optimization
During our initial attempt to carry out this study, our attempts to orally gavage the mice resulted in a level of mortality the led to the termination of this study. Following this, a number of alternative gavage needles were explored and a preferential smaller sized alternative was identified and is being successfully used in our current iteration of *Specific Aim 1*.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications.

  Nothing to Report

  Books or other non-periodical, one-time publications.

  Nothing to Report

  Other publications, conference papers, and presentations.

  Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Kathleen Rodgers
PI/Project Supervisor
ID #0157697
1.2 calendar months
Study design supervision, purchasing/timeline approval
Funding Support: National Institute of Health, US Biotest subaward, School of Pharmacy

Kevin Gaffney
Postdoc Scholar/Research Associate
ID #0238042
3.8 calendar months
Drug compounding and syringe preparation for initial animal studies.
Funding Support: USC Gift Account

Andrew Tiemann
Research Lab Technician 1
ID #2014924
6.0 calendar months
Animal handling technician. Fills syringes and delivers supplies from lab/receipt area to procedure room.

Michael Weinberg
Research Lab Technician 2
ID #2009562
9.0 calendar months
Animal/supplies purchasing, supply coordination, sterilizes instruments and provides technical help when requested.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.